Original Article

Immuno-histochemical Expression of Cyclin D1 in Oral Squamous Cell Carcinoma, Oral Potentially Malignant Disorders, and Normal Oral Mucosa

Shabana Bakhtiar*, Sabeen Nasir*, Sara Zia*, Hoor Maryam†, Natasha Kamran*, Karishma Ali*

1 Department of Oral Pathology, Peshawar Medical and Dental College, Peshawar (Affiliated with Riphah International University, Islamabad)
2 Department of Histopathology, Peshawar Medical College, Peshawar (Affiliated with Riphah International University, Islamabad)

ARTICLE INFO

Key Words: Oral Potentially Malignant Disorders, Oral Squamous Cell Carcinoma, Cyclin D1 Immuno-histochemistry


*Corresponding Author: Shabana Bakhtiar Peshawar Medical College, Peshawar, Pakistan wadaanmohmand@gmail.com

Received Date: 19th May, 2022
Acceptance Date: 26th May, 2022
Published Date: 31st May, 2022

ABSTRACT

Due to a high risk and tendency of OPMDs towards OSCC, its early detection is essential for better survival rate. Several molecular markers are available for diagnosis and prognostic assessments of OPMDs, also evaluating aggressiveness of OSCC. Objectives: To assess the immuno-histochemical expression of Cyclin D1 in OSCC, OPMDs, and normal healthy oral mucosa. Cyclin D1 has a significant role in cell cycle control and also strongly linked with the metastatic and poorly differentiated tumour cells. Methods: The study comprised of already diagnosed 20 cases of OSCC, 20 cases of OPMDs, and 20 normal oral mucosa cells, as a control. Results: Cyclin D1 immuno-reactivity was found positive in 100% cases of OPMDs, and 100% in OSCC but not in normal oral mucosa cells. Conclusion: A statistically significant expression of Cyclin D1 was observed in OPMDs which may indicate the probability of their transformation to OSCC.

INTRODUCTION

Oral cancer predominantly arises from epithelium cells in which 90 to 95% are diagnosed as Oral Squamous Cell Carcinoma (OSCC) [1]. OSCC is one of the 10th most prevalent malignancies worldwide, responsible for approximately 300,000 new cases and about 145,000 deaths on annual basis [2]. It is the most frequent reasons of death consisting about 50% of the entire cancers [3]. In the United States, 63,000 new cases of head and neck cancers are likely to occur in 2017 accounting approximately 4% of newly diagnosed cancers [4]. OSCC is among the most leading malignancies in South and Southeast Asian countries where, it is the second common malignancy in Pakistan (both in male and female) but first among males with 15.9% new cases and second after breast cancer in females [5,6]. The commonly affected sites of OSCC are tongue, floor of the mouth, and buccal mucosa [7]. The well-established risk factors involved in its development are tobacco use and aggressive intake of alcoholic beverages [8]. Other possible risk factors can be Human Papilloma Virus (HPV) infections and nutritional deficiencies, among others [9]. Tobacco can be consumed in chewable and non-chewable forms. In South Asia Chewable tobacco is more common in form of naswar, paan, and gutka, among others. In Pakistan, common habits of taking excess amount of areca nut or any sort of smokeless tobacco betel quid, gutka, pan masala, naswar,
Method

This descriptive cross-sectional research study was conducted in Pathology Department of Peshawar Medical Medical College, which consisted of histologically diagnosed 20 cases of OSCC and 20 cases of OPMDs. The samples were collected through the non-probability convenient sampling technique. Already diagnosed Formalin-Fixed Paraffin Embedded (FFPE) blocks were obtained from the Pakistan Institute of Medical Sciences (PIMS), Histopathology Department's archives. Patients with recurrent tumours and those undergoing chemotherapy or radiation were excluded. 20 normal healthy oral mucosa cases from the alveolar ridge were obtained from the patients coming to Peshawar Dental College for tooth extraction or other dental procedures, after taking informed consent from the patients. Before starting the study, Ethical approval was taken from the institutional review board under the approval No. Prime /IRB/2019-178 of Prime foundation. Cyclin D1 immunohistochemistry was done on sections with the maximum epithelial content after histopathological examination of various kinds of OPMDs and OSCC. The immunohistochemistry (IHC) slides were subdivided into five groups. Each batch contained one slide of Mantle cell lymphoma as a positive control. Antigen retrieval was accomplished using a microwave oven, during the procedure. For immunohistochemical labelling of selected sections, a primary antibody (monoclonal mouse antihuman Cyclin D1) and a secondary antibody (Dako EnVisionTM detection system) were applied. During procedure antigen retrieval was carried out using microwave oven. (Monoclonal Rabbit anti human CyclinD1) as primary antibody and (Dako EnVisionTM detection system) as secondary antibody were used for immunohistochemical staining of selected sections. Cyclin D1 immuno-positivity was assessed by the presence of brown colour immunostaining in the nucleus. Scoring criteria for Cyclin D1 immuno-positivity was categorized using scoring method prescribed by Dhingra, 2017 [17]. At 400X magnification, the expression of positive cells was assessed in at least five areas and then was graded as (1) 1-25%, (2) 26-50%, (3) 51-75%, and (4) >75%. Immunostaining intensity of Cyclin D1 was scored as (1) Mild, (2) Moderate, and (3) Strong staining. Total Scoring (TS) of CyclinD1 was calculated by multiplying Expression Score (ES) with Intensity Score(IS) to produce an Immuno-Reactivity Score (IRS), which was then graded as (1-4) mild, (5-8) moderate, and (9-12) strong. For the statistical analysis, statistical package for social sciences (SPSS) version 20.0 was used. P-value ≤ 0.05 was considered as statistically significant.

Results

Most of the patients age in OSCC and OPMDs were above 60 years, presenting with mean ages of 56.7 and 57.85 years, respectively. MF in OSCC was 1:1.2 while in OPMDs it was 2:3. Cyclin D1 expression was found positive in all layers of squamous epithelium in OSCC. In (80%) cases of OPMDs, Cyclin D1 staining was restricted to basal layer only while (20%) cases showed staining both in basal and supra-basal layers of squamous epithelium, Table 1.

<table>
<thead>
<tr>
<th>Staining</th>
<th>OSCC</th>
<th>WDCSCC</th>
<th>HDSCC</th>
<th>PDSCC</th>
<th>Total</th>
<th>Oral Leukoplakia</th>
<th>Oral Lichen Planus</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal Layer Only</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>8</td>
<td>8</td>
<td>16 (80%)</td>
</tr>
<tr>
<td>Basal and Supra-basal Layers</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>All Layers</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 (100%)</td>
</tr>
</tbody>
</table>

Table 1: Allocation of the cases according to groups and pattern of staining for Cyclin D1 immuno-reactivity.
The statistical comparison made among the 3 study groups (OSCC, OPMDs, and Normal Oral Mucosa) for Cyclin D1 immuno-reactivity showed significant results p value (<0.05), Table 2.

Table 2: Comparison of Cyclin D1 immuno-reactivity status between OSCC and OPMDs normal oral mucosa (*p value by Chi Square test)

Table 3 shows that in OSCC, all the 20 cases (100%) showed strong intensity. All 6 cases (100%) of Well Differentiated Squamous Cell Carcinoma (WDSCC) had strong Cyclin D1 staining intensity. All the 7 cases (100%) of Moderately Differentiated Squamous Cell Carcinoma (MDSCC) expressed strong Cyclin D1 staining. In the cases of Poorly Differentiated Squamous Cell Carcinoma (PDSCC), in all 7 cases (100%) the intensity of staining of Cyclin D1 was strong.

Table 3: Cyclin D1 staining intensity among the cases of OSCC

Table 4 explains that in all 20 cases of OPMDs, staining intensity of Cyclin D1 was weak in 7 cases (35%), and moderate in 13 cases. Mild staining intensity of Cyclin D1 was found in 4 (40%) cases, and moderate in 6 (60%) cases, out of 10 cases of Oral Lichen Planus. Out of 10 cases of Oral Lichen Planus the staining intensity of Cyclin D1 was observed to be weak in 3 (30%) and moderate in 7 (70%) cases.

Table 4: Cyclin D1 staining intensity among the cases of OPMDs

D I S C U S S I O N

In the current study, the most common age group of patients diagnosed with OSCC and OPMDs were over 60 years, with mean age in OSCC was 56.76 years. One such study in Pakistan found similar findings, with the average age of the sample population being 53.13 with a range of 25 to 80 years [18]. But contrary to our cross sectional study results (where the age group for OSCC cases was not limited), mean age of 48.35 years was reported with the age range of 20-85 years in a study conducted in India [19]. In our study, the observed mean age was 57.85 years in case of OPMDs, our results are supported by a research study, done in Brazil who reported that average age for OPMDs had been 60 years [20]. In contrast, a study conducted in Saudi Arabia, found the younger age range of (30-40 years) in case of OPMDs [21]. The differences in age groups can be due to small sample size of our study. Males are generally more affected from OSCC and OPMDs as compared to females. But in the present study, female showed pre-dominancy than males. In this study, M & F in OSCC was 1:1.2, comparable to our findings, studies done in Lebanon, Singapore and Sweden also found similar results in Bulgaria, Austria, Denmark, Ireland, and England [22]. In contrast to our findings, males constituted almost 75% of the study to determine the clinic-pathological significance of cyclin D1 in oral cancers [23]. In case of OPMDs, the male to female ratio in our study was 2:3, similar with Iranian research studies which showed female pre-dominancy [24]. A study done in Brazil in which (males constituted 78% while female constituted 59% of the study population) showing contrast to our findings [25]. In the present study, a statistically significant (p<0.05) (Table 1) relation was found between the immune-reactivity score of OSCC, OPMDs and Normal healthy oral mucosa. Similar strong relation of the cancer cells for expression of Cyclin D1 using immune-histochemical methods (to determine its expression in tumour specimens) was reported in a research study conducted in Tokyo, Japan [26]. In present study all the 20 cases of OSCC showed strong staining intensity for Cyclin D1 (Table 2). A study done in India almost showed similar results to our findings, demonstrated that most of the WDSCC cases (80%) showed strong Cyclin D1 staining intensity [27]. However, in contrast to our findings, another study from India demonstrated that 30% cases of OSCC showed mild to moderate intensity for Cyclin D1. In OPMDs, out of all 10 positive cases of leukoplakia, 40% cases showed mild while 60% cases showed moderate Cyclin D1 staining intensity. Contrary to our results, a study done in India found that 52.38% cases of oral leukoplakia showed mild while 33.33% showed moderate staining intensity for Cyclin D1 expression which showed that intensity of Cyclin D1 increases with decrease in severity of the lesion [28]. The discrepancy in staining intensity of Cyclin D1 can be due to large sample size of the Indian study. After extensive literature research no study could be found of Cyclin D1 expression in oral lichen planus. Similar to our study where the normal oral mucosa showed negative Cyclin D1 expression, a study conducted in India considered the expression of Cyclin D1 to be negative in normal cells i.e. <10% [29]. But in contrast to an another study done in India in which 60% cases of normal oral mucosa showed mild
staining of Cyclin D1 in basal and para-basal layers of squamous epithelium [27]. A larger sample size for research study in order to establish the effectiveness of Cyclin D1 is recommended. The study should also have follow up to assess role of Cyclin D1 in progression of OPMDs to OSCC as expression of Cyclin D1 was seen both in basal and supra-basal layers of OPMDs but was observed in all layers of OSCC. Thus Cyclin D1 expression significantly changed ROM oral epithelial dysplasia to OSCC. As oral cancer is mostly preceded by oral pre cancer so it species changed ROM oral epithelial dysplasia to OSCC. As oral cancer is mostly preceded by oral pre cancer so it species changed ROM oral epithelial dysplasia to OSCC. Thus Cyclin D1 expression significantly increased expression level of Cyclin D1 could be an early event in Oral cancer progression. Thus, Cyclin D1 marker might be valuable in assessing their prognosis and potential for malignancy.

CONCLUSION
Early detection of the transformation of OPMDs towards OSCC is necessary for the improvement of survival rate. Upon assessment of immune-histochemical expression of Cyclin D1 in OSCC, OPMDs, and normal oral mucosal cells, we were able to find positive expression of Cyclin D1 in OPMDs and OSCC but it didn't show any expression in normal oral mucosal cells. The results thus, indicate the probability of OPMD transformation into OSCC.

REFERENCES
quantitative analysis of immunohistochemical expression of p63, Ki-67, Cyclin-D1, and p16 in common oral potentially malignant disorders and oral squamous cell carcinoma. Journal of Dr. NTR University of Health Sciences. 2018 Apr; 7(2):120. Doi: 10.4103/JDRNTRUHS.JDRNTRUHS_13_18


